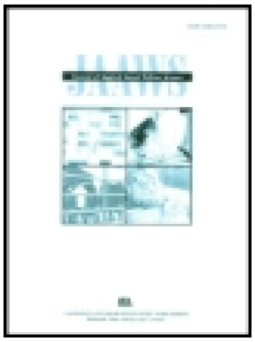

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The Use of a Species-Specific Health and Welfare Assessment Tool for the Giant Pacific Octopus, *Enteroctopus dofleini*

Meghan M. Holst[a] and Tim Miller-Morgan[b]

[a]Animal Care Department, Aquarium of the Bay, San Francisco, CA, USA; [b]Oregon Sea Grant/Carlson College of Veterinary Medicine, Hatfield Marine Science Center, Oregon State University, Newport, OR, USA

ABSTRACT
Cephalopods are increasingly viewed as sentient animals that require the same welfare consideration as their vertebrate counterparts. In this study, an observational welfare assessment tool developed by the EU Directive was revised to be species-specific for the giant Pacific octopus, Enteroctopus dofleini. This E. dofleini health and welfare assessment tool includes categories assessing E. dofleini external appearance, behavior, and clinical signs of stress and disease. These categories are scored in severity from 1 to 4, allowing a quantitative perspective on health observations. Six facilities used the health and welfare assessment tool to evaluate E. dofleini until the animal was humanely euthanized or died naturally. Results showed an irreversible upward trend in scores for feeding behavior and response to stimulus beginning 4 weeks prior to death, with significant changes in health and welfare scores between 4 weeks and the final week prior to death. This suggests that upward trends in these two variables predict death within 3–4 weeks. Highly variable results between individuals for other categories indicate that a quantitative tool can help assess health and welfare at the individual level.

KEYWORDS
Welfare; octopus; senescence; stress; disease

Introduction

Most concern and regulation for welfare remains isolated to "higher sentient" (ability to feel, perceive or experience subjectivity) vertebrate animals (Andrews, 2011; Mather, 2019). However, cephalopods are increasingly thought of as sentient invertebrates by caretakers of nonhuman-animals and that they require the same consideration as their vertebrate counterparts (Moltschaniwskyj et al., 2007). Cephalopods are mollusks in the class Cephalopoda that include octopuses, squid, cuttlefish and nautiluses. Their ability to display sentience has been well-documented, and cephalopods display clear examples of planning (Mather, 2019), casual reasoning and imagination (Mather & Dickel, 2017), learning (Zarrella, Ponte, Baldascino, & Fiorito, 2015) personalities and play (Mather & Anderson, 1993), fear, and exploration, (Mather, 2019). Arguably, their learning capabilities and plasticity of application for learned behaviors suggests evidence of a conscious mind (Mather, 2019). Cephalopods are common display animals in public aquaria, intriguing guests with their intelligence, camouflage capabilities, problem solving skills, and unique body morphology (Anderson, Wood, & Byrne, 2002). Cephalopods have been studied for both their central and peripheral nervous systems, particularly the giant axon (Crook & Walters, 2011; Fiorito et al., 2014; Williamson & Chrachri, 2004). Cephalopods continue to be popular display and laboratory animals. Consequently, there has been an increased demand for health and welfare

CONTACT Meghan M. Holst meghanmholst@gmail.com Animal Care Department, Aquarium of the Bay, San Francisco, CA 94133, USA

standards for these highly intelligent animals (Andrews, 2011; Bekoff, 2007; Broom, 2007; Mather & Anderson, 2007).

The definition of welfare has evolved over the past several decades. Originally welfare advocates and laws defined positive welfare as an animal's capability to perform natural behaviors such as moving to shade or being able to turn around within their enclosures (von Keyserlingk & Weary, 2017). In more recent years, welfare terms have included the animals' emotional and mental states (Bekoff, 2007; Broom, 2007). For example, enforcing positive welfare includes removing unnecessary fear, pain, or suffering (Fiorito et al., 2015; von Keyserlingk & Weary, 2017). Positive welfare can also include a healthy emotional and mental state (Crook & Walters, 2011; Mather & Anderson, 2007; Posner, 2000). Here welfare is defined as an animal's collective physical, mental, and emotional states over a period of time, and is measured on a continuum from poor to good (Association of Zoos & Aquariums, 2019). While it's often easy to assume that animals feel pain, we must first understand if animals have the mechanoreceptors capable of perceiving noxious stimuli, acutely and chronically. It wasn't until 2013 that a study first described the presence of mechanoreceptors in the peripheral nervous system of cephalopods (Crook, Hanlon, & Walters, 2013). Crook et al. (2013) found that not only do cephalopods feel noxious stimulus, but they can exhibit hypersensitivity to injuries over extended periods of time. It is important to clarify that nociception is the ability to sense harmful or potentially harmful stimulus, while "pain" is the emotional response to a nociceptive stimulus, which can vary by individual. While we cannot conclude from this study if cephalopods may suffer or experience pain in the same sense that vertebrates do, it is certainly reasonable to assume that injury can compromise the welfare of cephalopods. Even a minor injury can be reflected in morbidity and mortality, such as small fin or mantle abrasions (Hulet, Villoch, Hixon, & Hanlon, 1979; Seeley et al., 2016) and associated bacterial infections (Sherrill, Spelman, Reidel, & Montali, 2000). Therefore, any exposure to injury would implicate a change in the animal's welfare.

Several organizations have begun the groundwork for establishing and enforcing welfare standards for invertebrates maintained in laboratories, public aquariums and zoos. Most recently in 2015, the European Union set legal standards for invertebrate welfare, adopting general guidelines for use and defined welfare standards (Fiorito et al., 2015). Fiorito et al. (2015)'s publication was groundbreaking in its comprehensive recommendations for the care, use, and welfare protection of cephalopods. The EU Directive 2010/63/EU included cephalopods under the protection of animals for scientific purposes in 2015, setting the bar for invertebrate inclusive standards. In their publication, Fiorito et al. (2015) provided a compelling argument for the need to include cephalopods under welfare regulations. Within this publication, Fiorito et al. (2015) developed an observational matrix that scored cephalopod behaviors from 1 to 4, 1 being a positive, apparently healthy individual and 4 being a deteriorating animal that is experiencing compromised health and welfare. Objective observational tools are absolutely essential for the care of cephalopods in zoos, aquariums and laboratories. Invasive procedures often promote acute or chronic stress. Most cephalopods are extremely short-lived, therefore it is best to avoid inducing unnecessary stress which may compromise the long-term health of animals utilized for prolonged display or research. While Fiorito et al. (2015) established the basis for the first general health and welfare guidelines for cephalopods in captivity, species-specific guidelines and tools are still lacking.

In this study, the observational matrix developed by Fiorito et al. (2015) was modified to be specific for the giant Pacific octopus, *Enteroctopus dofleini* in zoos and public aquariums. This study highlights the need for species-specific criteria when governing the welfare of animals under human care and how institutions utilizing such species-specific criteria have used these tools to determine humane end of life care. This is particularly important when *E. dofleini* experience senescence, their terminal post-reproductive phase (Anderson et al., 2002). As with other semelparous animals, *E. dofleini* undergoing senescence often experience major skin deterioration, become anorectic, develop infections and exhibit stereotypic repetitive behaviors (Anderson et al., 2002; Mann, Martin, & Thiersche, 1970). Understanding the point at which senescence affects the welfare of *E. dofleini* is still under debate. However, using such quantitative tools to assist in the decision-making process regarding animal disposition can be a valuable addition to the often subjective management discussions. Utilizing objective criteria to assess health and welfare can aid in reducing bias or contradicting perspectives between decision making groups with often competing objectives. Examples of these competing objectives include senior managements aversion to an empty *E. dofleini* exhibit, expectations of the public to have an apparently healthy animal on exhibit, concern about not having a back-up display animal, caretaker concern for animal well-being, or incomplete experiments. The use of a semi-quantitative tool can allow these often opposing perspectives to be discussed utilizing and mutually agreed-upon framework to arrive at a welfare-driven decision.

It can be difficult to distinguish between clinical signs of stress, disease and senescence. This emphasizes the need to properly rule out disease and investigate possible etiologies of stress before concluding that *E. dofleini* are entering senescence. Difficulty in determining the difference between stress and senescence supports the need for quantitative tools to determine the severity of clinical signs, how they may compromise health and welfare, and determine how long an observation should persist before concluding an animal is senescent and ultimately terminal. This study uses a qualitative tool that allows caretakers of all experience levels to evaluate their animals and apply a quantitative score to their observations. This mixed-methods approach allows caretakers to incorporate highly variable qualitative observations of *E. dofleini* and apply a quantitative score for more effective evaluation of overall health and welfare. The aim of this study is to validate the use of a health and welfare assessment tool and aid *E. dofleini* caretakers in determining terminal cases of semelparous animals. While this is particularly needed for terminal and senescent *E. dofleini*, this tool will also aid caretakers in distinguishing acute stress versus chronic stress and/or irreversible decline. Distinguishing the severity of health and welfare will facilitate caretakers to make informed decisions in regard to *E. dofleini* husbandry, such as determining when the health of an animal may improve, or when to euthanize. Such tools are crucial for ensuring informed and humane care.

Materials and methods

Forty giant Pacific octopuses at participating public aquariums were regularly assessed utilizing the *Enteroctopus dofleini* health and welfare assessment tool (Table 1). Of the 40 individuals monitored, nine individuals from six different institutions senesced and died naturally or were euthanized.

Participating facilities were recruited at relevant conferences and through the Aquaticinfo Listerver, an international listserv shared between biologists at aquarium facilities. Any facility that had *E. dofleini* under their care were qualified to enter the study. Staff members that qualified to enter data had to be the primary caretaker of the animal (i.e., responsible for the animal at least five days a week), as primary caretakers have the most well-rounded understanding of the animals daily behavior and husbandry. There were no exclusion requirements for *E. dofleini* entered into the study; however, data analysis was restricted to animals that experienced senescence and eventually died within the timeframe of this study. Using the *E. dofleini* health and welfare assessment tool does not require training, but the contact information for the principal investigator was provided to all participants should they have any inquiries.

Seventeen facilities contributed data to this study by entering an online survey of background history of each *E. dofleini* in their care. Depending on the initial health of the animals that entered the study, facilities contributed between 4 and 46 weeks of data (i.e., some animals died within 4 weeks of entering the study, whereas other facilities are still actively entering data 46 weeks later at the time of publication). Background information was also collected and included the animal's unique ID, sex, collection origin, collection technique, and date the animal arrived at the participating facility. Participating facilities then used an online version of the *E. dofleini* health and welfare assessment tool (Table 1) every week to evaluate *E. dofleini* under their care. Table 2 is a one-page representation of the expanded Table 1 that caretakers can fill-out and keep on record (Table 2). Having a one-page summary allows caretakers to quickly

Table 1. *Enteroctopus dofleini* health and welfare assessment tool. This table is modeled after Fiorito et al. (2015) health matrix for all cephalopods. This matrix has been modified to be species specific for the giant Pacific octopus, *Enteroctopus dofleini*. Categories are listed in the left most column, and descriptions of health and welfare scores from 1–4 are read from left to right. A health and welfare score of 1 indicates a healthy individual exhibiting no signs of stress. A health and welfare score of 2 are acute signs that will typically resolve on their own. A health and welfare score of 3 are signs often reflective of chronic stress. A health and welfare score of 4 are severely chronic clinical signs of stress that will not mitigate on their own and most certainly compromise the animals welfare.

Categories Of Stress Response	**Score 1** No welfare concern	**Score 2** Monitor animal with increased frequency depending upon the parameter; increased WQ assessment	**Score 3** Monitor for signs of resolution or increased severity; seek advice and treat where possible.	**Score 4** Requires Immediate action including euthanasia when observed or at the end of a defined monitoring period.
External Appearance				
Skin Color	Normal skin color, pattern, appropriate/ prompt changes to external stimuli (prey, threat, conspecific, aquarist interaction, food, etc.)	Occasional inappropriate flashing, deimantic (threatening or startling) display in absence of an overt stimulus; transient pallor such as that seen during general anesthesia (reversible); unusual skin markings or coloration should always be monitored for changes with time.	Frequent abnormal displays; consistently displays white coloration unless provoked; uncoordinated color changes between arms, head or mantle; some continuously pallid areas or areas with an unchanging color or pattern (often associated with swelling or skin lesion).	Entire animal pale and fails to change color when challenged.
Skin Texture	Skin is smooth with a thin mucous layer except when there is a stimulus appropriate display of papillae; no swelling on body or arms	Small swelling (relative to the size of animal), not in a location that interferes with vision or feeding and with no breach of the skin. Occasional behaviorally unrelated display of papillae	Continuous display of papillae possibly indicative of an aroused state; Continuous smooth texture and display of papillae completely absent unless overtly provoked; larger swelling suddenly appearing; a small swelling increasing in size; swelling interfering with vision or ability to feed (buccal area); swelling with signs of potential infection (e.g., a fluid filled cyst); excessive skin mucus production	Swelling associated with breach of the skin; gas filled swelling likely to interfere with posture (not to be confused with gas trapped in the mantle cavity). Animal fails to display texture change when appropriate, including when provoked, moved, or stimulated in any way.

(*Continued*)

Table 1. (Continued).

Categories Of Stress Response	**Score 1** No welfare concern	**Score 2** Monitor animal with increased frequency depending upon the parameter; increased WQ assessment	**Score 3** Monitor for signs of resolution or increased severity; seek advice and treat where possible.	**Score 4** Requires Immediate action including euthanasia when observed or at the end of a defined monitoring period.
Skin Integrity	Skin intact (no underlying muscle exposed) over the entire body (dorsal and ventral surfaces).	Small, punctate breaches on arms or mantle (often caudal regions – "butt burn") showing a distinct adherent wound edge indicative of healing; no overt signs of infection.	Larger and more numerous breaches especially with irregular detached edges; small breaches that increase in size or develop a stable coloration distinct from adjacent skin; multiple small lesions occurring over the majority of the body	Full thickness (muscle visible and/or lesion has exposed viscera in the mantle cavity opening into the mantle cavity) skin lesions in multiple parts of the body (arms and mantle) covering >10% of apparent surface; wound dehiscence following a surgical procedure especially cranial or mantle if liver capsule opened because of gut herniation risk;
Abnormal Body Morphology/ Posture	Normal oppositional relationship between the arms, head and mantle appropriate to the location in the tank	Arms unaligned with mantle during jetting; limp arm while hanging on a wall and the ends of arms are not attaching to the substrate; minor arm curling at the ends that looks like a pig-tails	Arm with a permanent acute angle indicative of a muscle trauma; multiple arms limp at least halfway down arm and unable to adhere to substrate/ person/food; Extreme arm curling is observed on all arms; Limpness in arms resulting in lack of sucker adhesion on second half of arms.	Multiple complete arms limp while hanging on the wall; seems unable to appropriately grab objects or substrate; does not exhibit sucker adhesion on any arms, resulting in arm limpness.
Eyes	Normal prominent position; clear cornea and pupil diameter; orientation appropriate for light level and cranial axis	Unilateral clouding of cornea; nystagmus; rapid involuntary movements of the eyes.	Eyes sunken indicative of weight loss; pupils incorrectly oriented in relation to head; exophthalmos or enophthalmos.	Bilateral clouding of corneas (functionally blind – unresponsive to visual stimuli); fixed constricted or dilated pupils unresponsive ambient light change.
Number of arms	All arms, tentacles and suckers present and intact with no indication of regeneration	Part or all of one arm missing with signs of wound healing	Missing arm with no sign of healing; multiple arm loss with healing	More than 3 arms lost with no signs of healing

(*Continued*)

Table 1. (Continued).

Categories Of Stress Response	**Score 1** No welfare concern	**Score 2** Monitor animal with increased frequency depending upon the parameter; increased WQ assessment	**Score 3** Monitor for signs of resolution or increased severity; seek advice and treat where possible.	**Score 4** Requires Immediate action including euthanasia when observed or at the end of a defined monitoring period.
Animal Found Dead	N/A	N/A	N/A	Investigate immediately, including necropsy. An assessment should be made of the degree of suffering prior to death
Behavior				
Unprovoked behaviors				
Apathetic and/or withdrawn	Animal normally explores tank, is curious about novel objects in tank.	Reluctance to leave den/refuge area; rarely seen exploring tank	Has not been observed to leave den/refuge on seven consecutive days; adopts a defensive posture in den (often referred to as a "pumpkin" shape with suckers facing out and arms wrapped around head)	Does not leave den/ refuge even when challenged for a period of 3 weeks. Little to no reaction when provoked.
Abnormal body position in water column	Animal is able to maintain a position in the tank/water column with ease and to move in relation to a stimulus (e.g., food, light change, conspecific).	Mantle floating upwards (and mantle cannot be "burped"); animal continually swimming and appears to experience difficulty in maintaining a stable position in the water.	Prolonged periods of time with part or all of body out of water.	Octopus in a fixed location with most or all of its body out of the water.
Abnormal motor or locomotor coordination	Locomotion and other motor activity (e.g., prey capture) is precisely coordinated.	Inability to coordinate arms/body on 2 consecutive occasions; inability to maintain a straight line; persistent tremor/ twitching in limbs; occasional jetting into walls or uncoordinated swimming, might cause a central mantle receding or "butt burn"	Further deterioration or resolution in 48 hours; stiff movement; bradykinesia; ataxia. Daily but not continuous jetting and uncoordinated swimming; frequently running into walls likely causing multiple mantle lesions	Convulsions, seizures, or extensive muscle spasms. Continuous uncoordinated swimming or jetting with frequent running into walls likely leading to multiple mantle lesions
Stereotypic behavior (chronic pacing, escape jetting, etc.)	Normal diversity of behavior with no indication of repetitive, overtly purposeless activity	Occasional repetitive, overtly purposeless activity (e.g., pacing, uncoordinated swimming, escape jetting, rubbing mantle on walls). Enrichment, food, or foreign object introduction can break this behavior.	Daily but not continuous repetitive, overtly purposeless activity; Enrichment, food, or foreign object can break this behavior at least every other day.	Continuous repetitive, overtly purposeless activity present (irrespective of when the animal is observed); enrichment, food, or foreign object cannot break behavior for 3 consecutive days.

(*Continued*)

Table 1. (Continued).

Categories Of Stress Response	**Score 1** No welfare concern	**Score 2** Monitor animal with increased frequency depending upon the parameter; increased WQ assessment	**Score 3** Monitor for signs of resolution or increased severity; seek advice and treat where possible.	**Score 4** Requires Immediate action including euthanasia when observed or at the end of a defined monitoring period.
Grooming/Cleaning	Cleaning behavior is most obvious in octopus that can reach all parts of the body with arms and is a normal periodic activity; sucker rings floating in the water may be a surrogate marker for grooming.	Animal spends progressively more time demonstrating grooming behavior or signs that grooming is reduced leading to a deterioration in skin condition or obvious rings hanging from suckers	Continuous grooming when presented with food or a distraction; mucus accumulation; skin infection or algal deposits may be a marker of significantly reduced grooming	Complete absence of grooming or continuous grooming indicated by continuous wiping of mantle by the arms in octopus
*Inking	Does not ink, or only inks as a response to an immediate threat.	On consecutive days animal inks when the tank is opened or human is visible (wearing dark clothing)	Inking behavior happens three times in the same week period	Persistent inking every time a stimulus is introduced (e.g., presence of human); should not occur for three days in a row, and should not persist more than four times within a 7 day period
*Egg laying/sperm packet deposition	Has not deposited gametes	N/A	N/A	Has deposited gametes
*Autophagy	Not observed – autophagy is not a normal behavior so any occurrence of autophagy should be investigated promptly	Removal of a few suckers or a skin lesion on the arm may indicate incipient autophagy	Removal of a few suckers or a skin lesion on the arm on two separate occasions	Clear intentional arm chewing; removal of an entire arm or multiple arms; exposed muscle tissue from chewing
Wound/lesion directed behavior	Might only occur after a procedure or recent injury.	Probes wound occasionally in first 24 hours post procedure; with an arm lesion may examine arm with mouth	One or more arms continuously in contact with wound or attempting to reach wound in 24 hours post procedure	One or more arms continuously in contact with wound in 24 hours post procedure and wound shows signs of dehiscence or infection; treatment does not seem to promote healing; animal starts chewing around wound.
Provoked Behaviors				
Defecation	Normal defecation, not typically provoked by a stressor.	One suspected incidence of stress related defecation	More than one suspected incidence of stress related defecation, but incidents occur more than a week apart	More than one suspected incidence of stress related defecation within the same week

(*Continued*)

Table 1. (Continued).

Categories Of Stress Response	**Score 1** No welfare concern	**Score 2** Monitor animal with increased frequency depending upon the parameter; increased WQ assessment	**Score 3** Monitor for signs of resolution or increased severity; seek advice and treat where possible.	**Score 4** Requires Immediate action including euthanasia when observed or at the end of a defined monitoring period.
Inking	Inking is part of a defensive response, and Giant Pacific Octopus should not ink as a healthy response to provoked stimuli or human presence unless the provoked stimulus is threatening	Octopus inks as a result of a single provoked stimulus, novel stimulus, or clear stressor. This is often a display of acute stress that can be mitigated.	Inking occurs on three separate occasions within 7 days from a provoked stimulus (e.g., human touch), or animal inks when seemingly unprovoked. This is a display of chronic stress.	Persistent inking every time a stimulus is introduced (e.g., presence of human) and mitigation does not resolve the behavior; Should not occur for three days in a row, and should not persist more than four times within a 7 day period
Response to stimuli (humans and non-food items placed in the tank) *(see also withdrawal or apathy)*	Animal readily responds to human or object entering room or enclosure, often displayed by the animal tensing up, texturing up, or camouflaging; animal may reach out to investigate human or objects in reach, frequently pulling foreign objects toward the mouth; novel items are readily explored	Occasionally fails to respond to the appearance of human or novel non-food object, but will still readily respond with direct contact	Fails to respond to stimulus unless there is direct physical contact, but even then reaction to stimulus is subdued; apparent aggressive behavior indicated by directed squirting at human; withdraws to den on appearance of human	No response to stimulus, even when provoked by physical contact; may move arm, but does not suction onto or grab stimulus when there is direct contact
Color and texture change	Cephalopod color and texture normally changes throughout the day, especially during feeding, interaction, and exploration	Absence of color or texture change during activities where color change is normally observed	Absence of color or texture change, even when provoked with food, novel items, and human interaction	No color or texture change for 7 days, even when provoked with food, novel items, and human interaction; often correlated with withdrawal/apathy
Defensive behavior	Not observed – Cephalopods are normally curious at novel objects, and typically do not aggressively withdraw from the presence of humans or novel objects	Signs of withdrawal or defense from human or object, seen by pulling arms away, jetting away or actively avoiding	Actively escape jets or withdraws arms from contact (often looking “pumpkin shaped”) on 2 consecutive days	Actively escape jets or withdraws arms from contact (often looking “pumpkin shaped) for 3 consecutive days
Feeding Behavior				

(*Continued*)

Table 1. (Continued).

Categories Of Stress Response	**Score 1** No welfare concern	**Score 2** Monitor animal with increased frequency depending upon the parameter; increased WQ assessment	**Score 3** Monitor for signs of resolution or increased severity; seek advice and treat where possible.	**Score 4** Requires Immediate action including euthanasia when observed or at the end of a defined monitoring period.
Feeding Behavior	Rapidly approaches and captures/takes food; attack latency within 1 SD of the normal range established for a given lab/species/prey type; for live prey attack should be coordinated and prey subdued quickly.	Reduced urge; increase time to attack	Progressive increase in attack latency and uncoordinated attack; misses target or will not attack	No desire to attack or unable to subdue live prey; refuses food multiple days in a row even when food is placed directly in the animal's arms
Clinical Signs				
Digestive				
Food intake	Readily takes and consumes food and exhibits a willingness to work for the food. For example, a healthy animal will readily solve food puzzles to retrieve their food.	Fails to take food on two consecutive days (*assumes a daily or every two days feeding schedule)* or fails to completely eat a normal size meal.	Fails to take any food on 3 consecutive days including an attempt with a novel food or live prey if animals not normally given live prey.	Fails to take any food on 4 consecutive days including when pieces of food are placed in the arms/near mouth.
Fecal output	Likely to be a large normal range. Feces should reflect proportional food intake. If an animal is not eating, it would normal for fecal output to be absent.	Reduced fecal output while food intake is normal	Only evidence of fecal output once every 1–2 weeks while still feeding regularly; presence of occasional parasites or cysts; cytological markers of epithelial damage.	Absent for a period of two weeks while animal is eating (may indicate gut obstruction); persistent presence of parasites or cysts
Vomiting/regurgitation	Not present – Not normally present so any occurrences should be a cause for concern but controversy over existence.	Occurs occasionally	Often occurs following ingestion of food	Always occurs following ingestion of food. If regurgitation occurs three consecutive days, action must be taken.
Rates				
Ventilation rate	Normal respiration (this may take time to establish). Average resting respiration should range between 8–12 breaths/minute, depending on enclosure temperature and animal activity	Increased respiration not accounted for from increased activity or anoxic event; respiration consistently decreased even when active	Abnormal respiration rate for two consecutive days; consistent respiration below 4 breaths/minute should be closely monitored and investigated.	Abnormal respiration rate for 3 + consecutive days
Growth Rate	Maintenance of normal growth depending on food availability, season, species, age, reproductive status.	No growth occurs even with sufficient caloric intake	10% loss of body weight over 1 month	20% loss of body weight over 1 month

reference progression of clinical signs and easily visualize changes in welfare scores. Institutions followed *E. dofleini* under their care every week for a minimum of six months, or until the animal was euthanized or died naturally. Animals were determined as senescent at each institution based on sexual maturity, gamete deposition, postmortem histopathology results, and/or historical data. Acquisition and subject characteristics of the nine *E. dofleini* that were analyzed are provided in Table 3.

In addition to filling out a weekly health survey, participating facilities recorded each week the diet, number of times the animal received enrichment, number of times the animal actively participated in enrichment, diversity of enrichment, how many days the animal was offered food, how many days the animal at least partially consumed food, the animal's exact weight (in kg), the system volume (in L), and the water quality parameters including ionic ammonia (NH_3 in mg/l), nitrite (NO_2 in mg/l) nitrate (NO_3 in mg/l) salinity (in ppt), temperature (in Celsius), and pH.

Finally, all caretakers that participated in this study were sent a survey evaluating the effectiveness of the *E. dofleini* health and welfare assessment tool. Thirteen institutions and 29 caretakers used the *E. dofleini* health and welfare assessment tool during this study.

Data analysis and statistical procedures

After data were recorded from participating facilities, severity scores were entered into Prism 8.4.0 (Graphpad Software, San Diego, CA) by category (i.e., Skin Color, Skin Texture, Skin Integrity, etc.), and collated into "weeks from death" intervals. Numerical scores were averaged across animals, and changes in observation severity (welfare scores 1–4) were tested for significance ($P < 0.05$) changes across time using a Kruskal-Wallis test.

Results

Of the 40 animals that were entered into the study, nine animals from six institutions ultimately died due to senescence. This decline was documented in the weekly health and welfare assessments. Six of these nine animals were observed for over 10 weeks (max = 47 weeks), while three animals died in less than 10 weeks of assessments (four, seven, and eight weeks). No significant changes were observed for any category before the last 10 weeks preceding death (p-values varied, but all were greater than $p > 0.08$). Therefore, only the last 10 weeks of assessments leading up to the animal's death were used for testing. For these nine individuals, all animals were displaying clinical signs for senescence within the last 10 weeks of their life and were deemed senescent during this time.

Two assessment categories were observed to have significant changes in observation leading up to death: feeding behavior and response to stimulus. Feeding behavior is defined as the animals behavior toward available food. Normally, *E. dofleini* will readily investigate known food sources or prey items. However, as they enter senescence this behavior will drastically change and eventually cease, as they become anorectic during senescence (Anderson et al., 2002). Response to stimulus is defined as any change in color, texture, or body movement as a response to an outside stimulus such as direct touch, or an approaching foreign object such as a feed stick or toy. Feeding behavior showed a significant change between four weeks prior to death to the time of death ($p = 0.02$) (Figure 1). Response to stimulus also showed a significant change between four weeks prior to death to the time of death ($p = 0.03$) (Figure 2). While changes were seen in many assessment categories including stereotypic repetitive behaviors, skin integrity, skin texture, and being apathetic and withdrawn, no other significant changes were seen consistently across all *E. dofleini* in the study (all p-values >0.05).

All scores for all categories were graphed for each individual *E. dofleini* for the last weeks leading up to death (Figures 3–11). These figures provide a visual representation of how highly variable observations were between individuals.

Table 2. This table is a one-page compliment to the *E. dofleini* health assessment tool. Using this page can be particularly helpful to visualize all of the observations the caretaker makes. A single-page compliment also allows for efficient record keeping should caretakers like to keep health assessments on record.

Date:	**Facility:**	**Animal ID:**				
Animal's gender:	Date animal came to facility:	Animal origin:		Times fed a week:		Diet (est. % of each food item):
Tank parameters (i.e., temp, size):		Water quality:	NH3:	NO2:	NO3:	
	Signs of Stress Response	**1/Normal**	**2**	**3**	**4**	**Comments**
External Appearance						
	Skin Color					
	Skin Texture					
	Skin Integrity (i.e., mantle receding/lesions)					
	Abnormal Body Morphology					
	Eyes					
	Number of arms					
Behavior						
Unprovoked Behavior						
	Apathetic and/or withdrawn					
	Abnormal body position in water column					
	Abnormal motor or locomotor coordination					
	Stereotypic behavior (chronic pacing, escape jetting, etc.)					
	Grooming/Cleaning					
	Inking					
	Egg laying/Sperm packet deposition					
	Autophagy					
Provoked Behavior						
	Defecation (increased or uncontrolled defecation while handling)					
	Inking					
	Response to stimuli (human and non-food items)					
	Color and texture change					
	Defensive behavior					
Feeding Behavior						
	Changed urge/speed to attack prey					
Indicates immediate suspension of additional stress factors (e.g., encounters) and requires immediate action regardless of severity						
Clinical signs						
digestive						
	Food intake					
	Fecal output					
	Vomiting/regurgitation					
Rates						
	Ventilation rate					
	Heart rate					
Other						
	Body weight					
	Blood Biomarkers					
Free Observation						

Table 3. Anonymized summary of analyzed *Enteroctopus dofleini*. Nine *Enteroctopus dofleini* experienced terminal post-reproductive decline during this study and their health and welfare scores were analyzed. This is a summary of all analyzed *E. dofleini*. There were a total of six institutions, and nine individual *E. dofleini*. All animals were wild caught by either scientific collector (SC) or fishermen (F). Enclosure volume represents the physical space that *E. dofleini* had access to, whereas system volume indicates the total body of water within the system (including sumps and filtration for closed systems). Enclosure and system volume changed for some individuals during the study if an animal was moved between enclosures, indicated with an asterisk. The volumes listed here were from the enclosures that the animal spend the majority of its time during the study.

Facility	Animal	Animal's Sex	Animal's Origin	Collected via Fishermen (F) or Scientific Collector (SC)	Weight Range During Study (kg)	Enclosure volume (L)	System volume (L)	Enclosure Temperature Range (C)	Weeks Observed
1	A	Male	Half Moon Bay, California, USA	Wild Caught (F)	7.6–17	717	717	12.2–15.7	17
1	B	Male	Bodega Bay, California, USA	Wild Caught (F)	29.48–34	1351392*	1351392*	12.7–16.1	15
1	C	Female	Vancouver, CAN	Wild Caught (SC)	6.8–12	2717.93	4555.74	7.8–9	38
2	D	Male	Vancouver, CAN	Wild Caught (SC)	4.54–5.44	3785	4542	9.44–10	24
2	E	Female	Vancouver, CAN	Wild Caught (SC)	4.54–5.44	1892.706*	2649.788*	10–12.22	30
3	F	Female	British Columbia, CAN	Wild Caught (SC)	4.5	4080	406000	9.4–12.8	31
4	G	Female	Vancouver, CAN	Wild Caught (SC)	17.25–18.14	4543*	5300*	10	5.5
5	H	Female	Vancouver, CAN	Wild Caught (SC)	6.7–12.1	1135*	1287*	9.8–11.7	33
6	I	Female	Vancouver, CAN	Wild Caught (SC)	1.5–6.4	6435	6435	8.9–10	45

Discussion

This study is the first of its kind to have facilities holding *E. dofleini* document extensive health and welfare assessments, general observations and husbandry practices over an extended period of time. While other studies have used records to retrospectively assess the health of *E. dofleini* (Seeley et al., 2016), none have documented health changes in real time among multiple facilities. While it is not surprising to most *E. dofleini* caretakers that changes in feeding behavior and response to stimulus would indicate terminal decline and senescence (Anderson et al., 2002), this is the first study to document a timeline of these behavioral changes associated with senescence and death. One aspect that should be further investigated in future research is the relatedness between response to stimulus and feeding behavior. This study did not remove feed sticks or food toys as a stimulus to *E. dofleini*, and therefore these variables could be correlated. Future studies should remove food-related stimulus from this category.

In addition to the two variables that yielded significant changes when all animals' scores were averaged, changes were seen in many categories for each animal, but no other categories yielded significant change across the entire population in this study. While many observations are seen consistently, such as anorectic behavior, stereotypic behavior, and loss of skin integrity, the results of this study indicate that the timing and sequence of events during senescent decline is highly variable between individuals. Additionally, many observations are measured with variable time scales. This coincides with our definition of welfare, which is a continuum of poor to good over a period of time. Caretakers must take into consideration where individual animals fall on the continuum and take into consideration all observations leading up to the present moment. Some observations become more severe over weeks (such as decreased food intake), whereas other observations become more

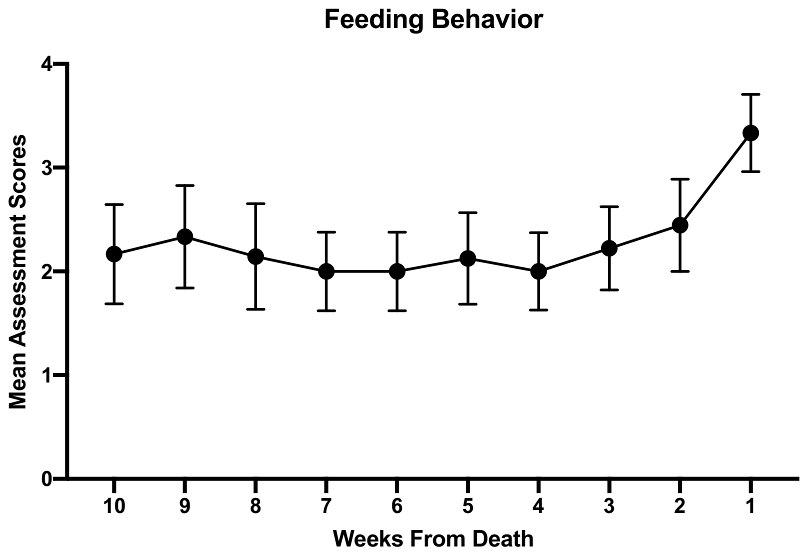

Figure 1. Health and welfare assessment scores 1(no/low welfare concern) – 4 (high welfare concern) of the last 10 weeks of the animals life were averaged and graphed. There was a significant change in feeding behavior between 4 weeks from death and the last week of the animals life ($p = 0.02$).

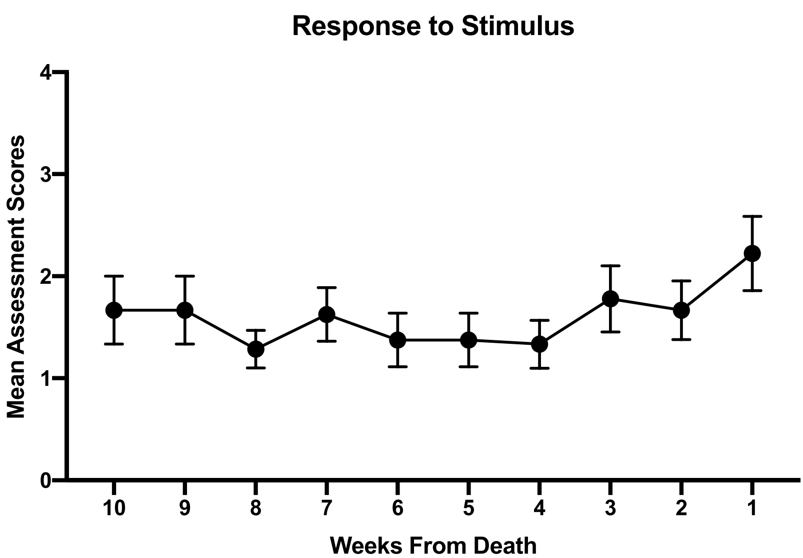

Figure 2. Health and welfare assessment scores 1 (no/low welfare concern) – 4 (high welfare concern) of the last 10 weeks of the animals life were averaged and graphed. There was a significant change in response to stimulus between 4 weeks from death and the last week of the animals life ($p = 0.03$).

severe in a matter of minutes (such as autophagy). Such variability highlights the need for objective, quantitative tools that provide perspective for highly variable observations for a single species in order to make well-informed welfare decisions.

While the health and welfare assessment tool is comprehensive of our current understanding of *E. dofleini*, future studies are still needed for clinical evaluation of animal health. Areas that should

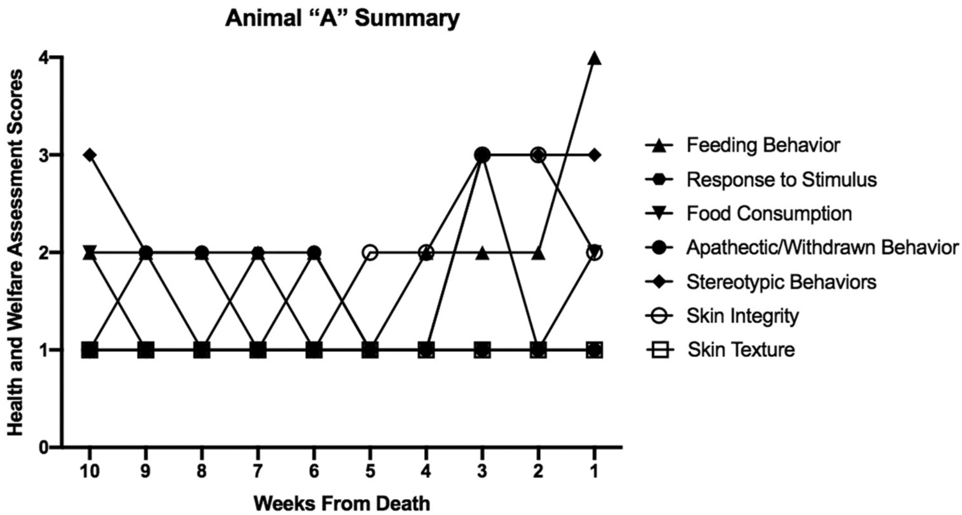

Figure 3. Health and welfare assessment scores 1(no/low welfare concern) – 4 (high welfare concern) of the last 10 weeks of animal "A"'s life was graphed for all behaviors.

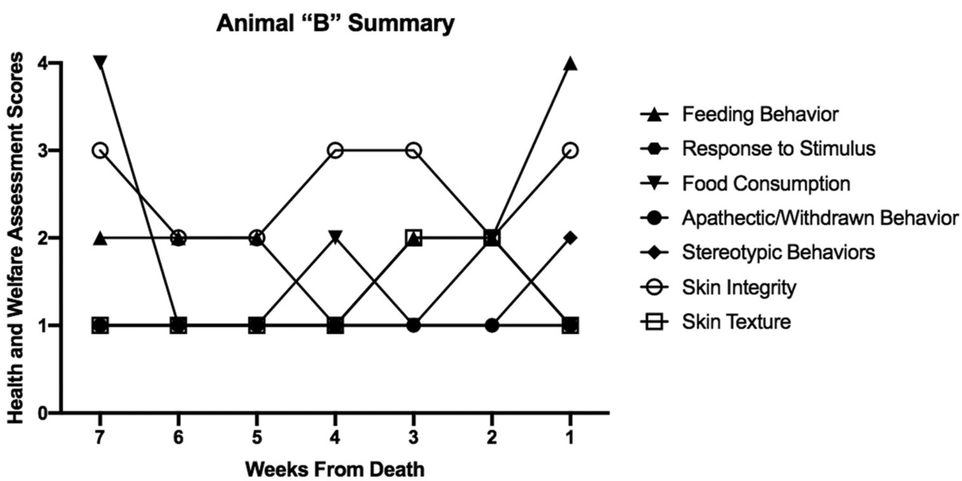

Figure 4. Health and welfare assessment scores 1(no/low welfare concern) – 4 (high welfare concern) of the last 10 weeks of animal "B"'s life was graphed for all behaviors.

be further investigated included ultrasound studies to evaluate heart rates and blood analysis to investigate biomarkers that reflect clinical health.

Each participating facility that used this health and welfare assessment tool were asked to fill-out an anonymous end-of-study survey. Only 10 of the 29 participants completed the anonymous survey, which may be correlated by individuals leaving their host institutions, or lack of availability to participate during the COVID-19 pandemic. Of those that responded, they all indicated that having a quantitative perspective aided in their decisions regarding the end-of-life care for *E. dofleini*. They also indicated that the tool typically validated their perspective and ultimately supported their husbandry decisions.

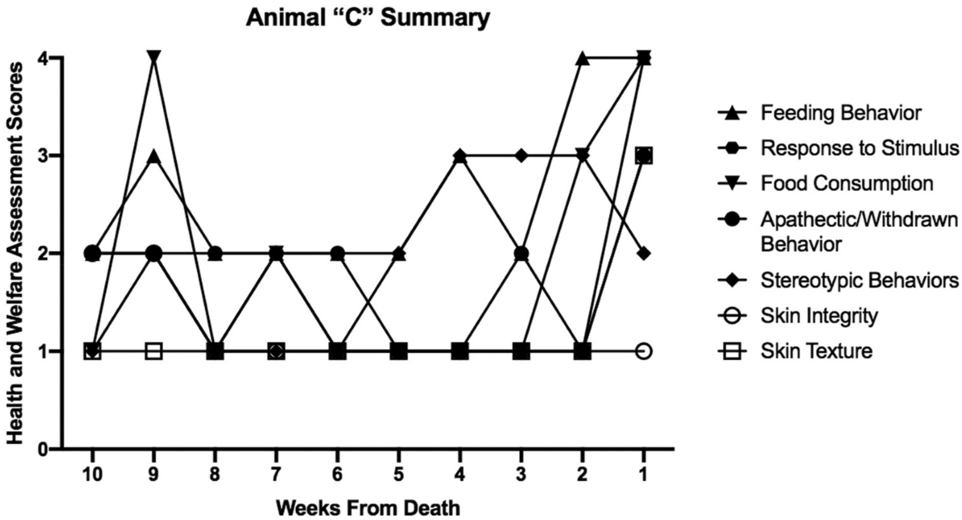

Figure 5. Health and welfare assessment scores 1(no/low welfare concern) – 4 (high welfare concern) of the last 10 weeks of animal "C"'s life was graphed for all behaviors.

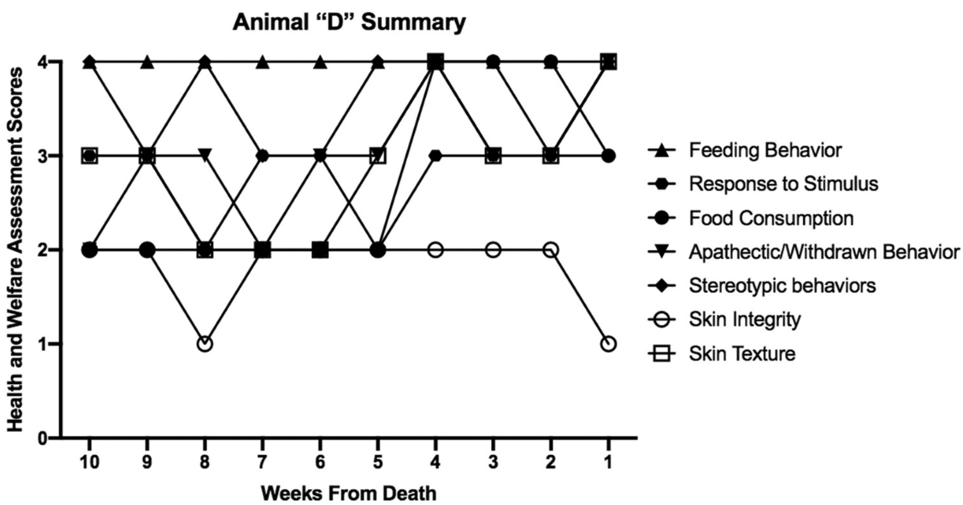

Figure 6. Health and welfare assessment scores 1(no/low welfare concern) – 4 (high welfare concern) of the last 10 weeks of animal "D"'s life was graphed for all behaviors.

While participants indicated that the health and welfare assessment tool benefited their husbandry decisions, participants were unsure on whether the same animal would be scored the same way by different biologists. This is reflective of the fact that the health and welfare assessment tool is not meant to replace the subjective and skilled viewpoint of professional caretakers, but to provide perspective, validate, and ultimately empower caretakers in their husbandry decisions. Such validation of the caretakers husbandry decisions will ultimately benefit the animals' care, particularly with difficult decisions such as euthanasia. While the purpose of the health and welfare assessment tool is to support the intuition of professional caretakers, a weakness of this study is the room for subjectivity when scoring. Future studies should investigate inter-rater dependency and validate

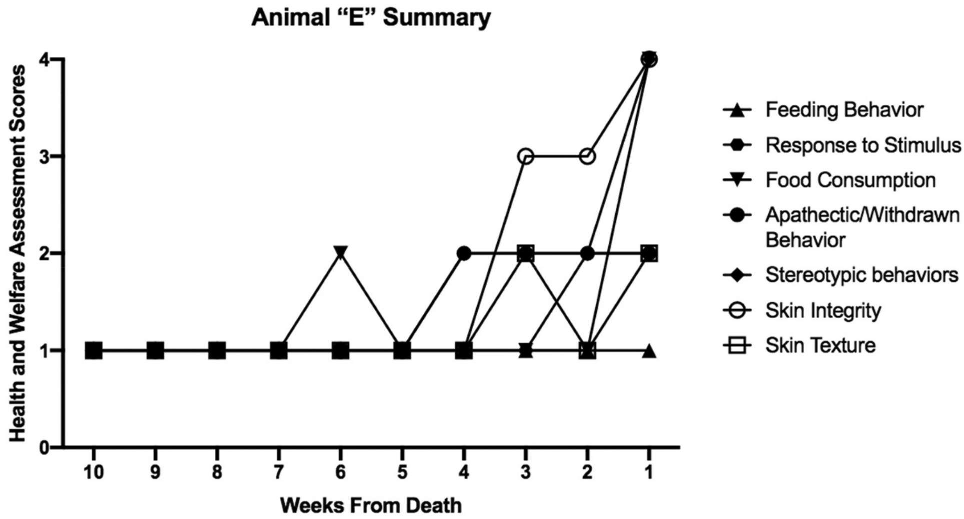

Figure 7. Health and welfare assessment scores 1(no/low welfare concern) – 4 (high welfare concern) of the last 10 weeks of animal "E"'s life was graphed for all behaviors.

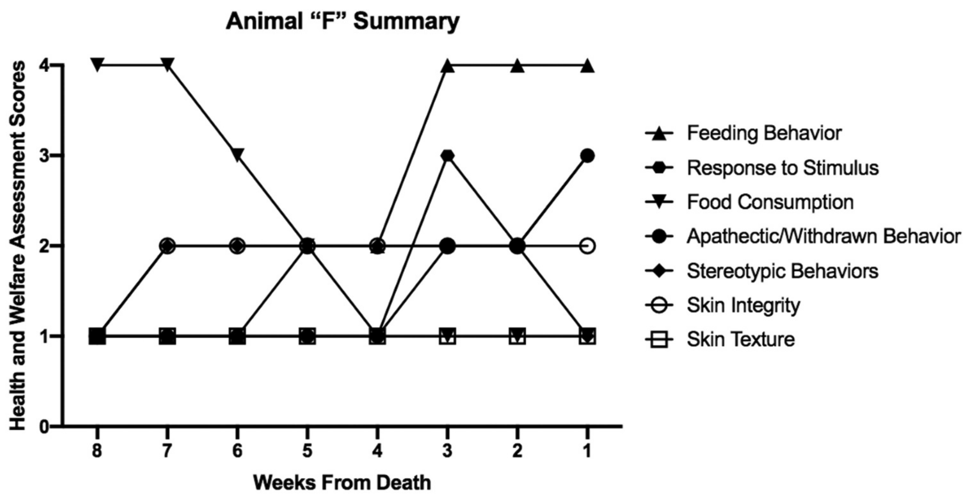

Figure 8. Health and welfare assessment scores 1(no/low welfare concern) – 4 (high welfare concern) of the last 8 weeks of animal "F"'s life was graphed for all behaviors. Animal "F" was not observed for 10 weeks because the animal died after 8 weeks of observations.

the repeatability of the score outcomes. Some studies have investigated body-scoring criteria (Clark, Pandolfo, Marshall, Mitra, & Schech, 2018). Validated scoring tools such as these would enhance to overall understanding of the health and welfare of *E. dofleini*.

The results of this study indicate that the *E. dofleini* assessment tool is an effective observational and quantitative health and welfare assessment tool that empowers animal caretakers. Clinical signs of terminal decline are highly variable and can be difficult to objectively assess and discuss, particularly when euthanasia is under consideration. For cephalopod experts, such observational quantitative tools provide a framework for all parties involved in animal welfare decisions to agree upon a standard. For cephalopod husbandry neophytes, this health and welfare assessment tool

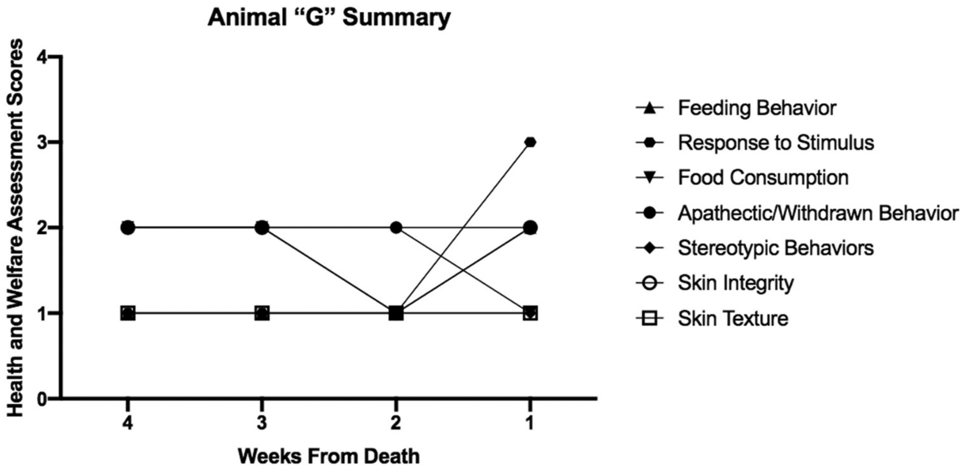

Figure 9. Health and welfare assessment scores 1(no/low welfare concern) – 4 (high welfare concern) of the last 4 weeks of animal "G"'s life was graphed for all behaviors. Animal "G" was not observed for 4 weeks because the animal died after 4 weeks of observations.

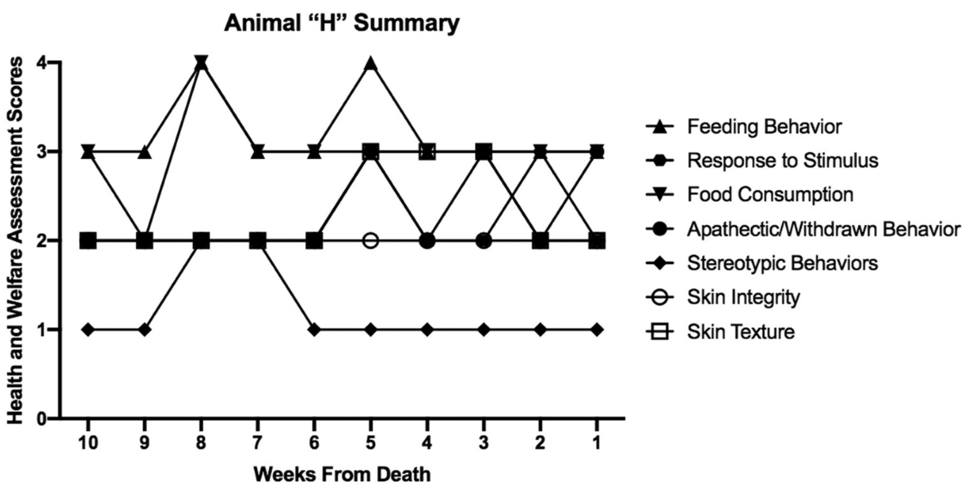

Figure 10. Health and welfare assessment scores 1(no/low welfare concern) – 4 (high welfare concern) of the last 10 weeks of animal "H"'s life was graphed for all behaviors.

provides basic guidelines that help them identify signs of acute, chronic, or senescence related stress in the *E. dofleini*. Quantitative tools assist all *E. dofleini* caretakers in gaining perspective on the severity and significance of changes in health and behavior, and provide a framework for assessing how these changes may impact an animals' welfare. While many of these observations are often reflective of senescence, acute or chronic stress, infectious disease, parasitism, exposure to contaminants and trauma may elicit similar observations. Quantitative tools provide perspective of an animals' state, and it is important to evaluate all possible contributing factors that may compromise the animals' welfare.

The welfare tool not only empowers caretakers in terminal care decisions, but more abrupt and potentially reversible welfare concerns. Based on the application of the *E. dofleini* health and welfare assessment tool, acute and reversible clinical signs of stress typically receive a welfare score of 2. For

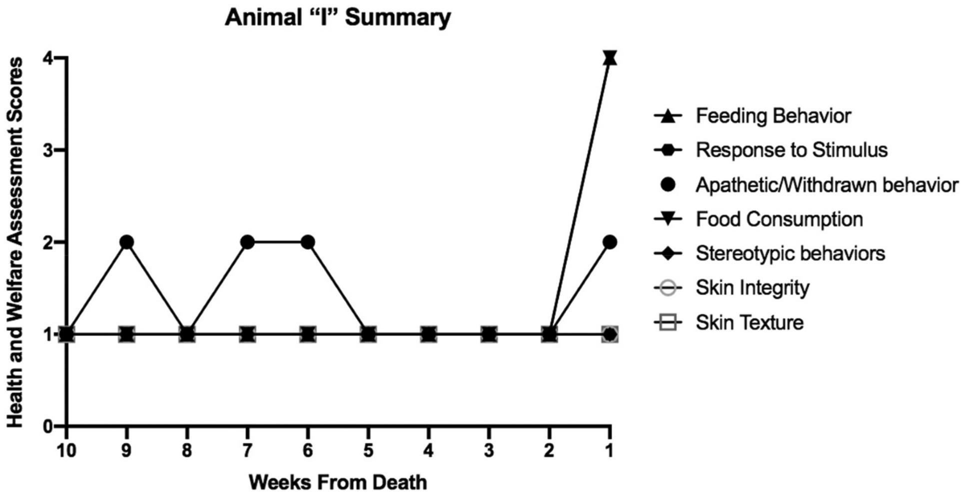

Figure 11. Health and welfare assessment scores 1(no/low welfare concern) – 4 (high welfare concern) of the last 10 weeks of animal "I"'s life was graphed for all behaviors.

example, poor water quality may result in increased grooming behavior, but will resolve back to a welfare score of 1 once water quality has improved. Acute clinical signs might receive a welfare score of 3, and gone unresolved might become chronic clinical signs that could progress to a welfare score of 4 if the eliciting factor is not mitigated. Persistent scores of 3 in the welfare category should certainly raise a concern about *E. dofleini* welfare and the animal should be closely monitored and husbandry factor corrected. Finally, we suggest that any welfare scores of 4 would indicate a serious compromise in animal welfare, and would encourage immediate mitigation. Based on our experience and observations, we also suggest that three welfare scores of 3 would equate to a single welfare score of 4. Any welfare score of 4 should be closely monitored and mitigated if at all possible. Persistent health and welfare scores of 4 indicate the need for serious discussion of euthanasia, as euthanasia in such cases is in the interest of good welfare.

This tool is specifically designed to measure welfare when there are concerns present. The field would benefit from future research that can differentiate between "no welfare concern" and "positive" welfare. For example, an animal may not exhibit any symptoms of stress, but also may not exhibit positive forms of welfare such as engaging in enrichment or learning opportunities. As such, this tool is not an effective evaluation for scoring positive health. Additionally, there are several unknown areas of *E. dofleini* physiology that should be further investigated, such as evaluating normal heart rates or investigating blood values. The field would also benefit from investigating health and longevity between animals based on enrichment or enclosure parameters.

Whether an animal is experiencing acute, chronic, or senescent related stress, it is pivotal that caretakers are able to understand the severity of the clinical signs and how they may affect *E. dofleini* health and welfare. The decision-making process regarding the management and/or disposition of a particular animal among husbandry professionals and facility administrators can become contentious when there is a disagreement regarding the severity of the signs. Such disagreements may lead to a delay in addressing the current needs of the specific animal. The *E. dofleini* health and welfare assessment tool provides objective criteria for assessing the severity of health observations, allowing a decision-making process that focuses on developing action plans that address the animals' current needs and ensures the best standard of care. Future welfare studies should continue developing species-specific quantitative tools that support institutional welfare standards.

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